Working standard solution. Dissolve approximately 100 milligrams of the cefotiam working standard, accurately weighed, in water and dilute to 100 milliliters. Further dilute with mobile phase to obtain a solution containing 50 micrograms of cefotiam activity per milliliter.

(B) *Sample solution.* Dissolve approximately 100 milligrams of the sample, accurately weighed, in water and dilute to 100 milliliters. Further dilute with mobile phase to obtain a solution containing 50 micrograms of cefotiam activity per milliliter (estimated).

(C) *Resolution test solution.* Dissolve an accurately weighed portion of cefotiam working standard in water to obtain a solution containing approximately 1.0 milligram of cefotiam activity per milliliter. Heat this solution at 95 °C for 15 minutes. This procedure allows cefotiam lactone to be produced. Dilute 1.0 milliliter of this solution to 100 milliliters with mobile phase.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (T) for the cefotiam peak is satisfactory if it is not more than 1.76 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 1985 theoretical plates for the cefotiam peak.

(C) *Resolution factor.* The resolution factor (R) between the peak for cefotiam and the peak for cefotiam lactone (generated in situ) is satisfactory if it is not less than 4.0.

(D) *Coefficient of variation.* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 1.0 percent. If the system suitability parameters have been met, then proceed as described in §436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of cefotiam per milligram of sample as follows:

$$\text{Micrograms of cefotiam per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cefotiam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefotiam peak in the chromatogram of the cefotiam working standard;

P_s=Cefotiam activity in the cefotiam working standard solution in micrograms per milliliter;

C_u=Milligrams of the sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(g) of this chapter, using a solution containing 40 milligrams per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter, using the sample preparation described in paragraph (d)(4) of that section and the titration procedure described in paragraph (e)(3) of that section, except:

(i) In lieu of 3 milliliters of anhydrous methanol solution, inject 20 milliliters of a formamide:methanol solution (2:1) into the container and shake to dissolve the contents (prior to use in preparation of the formamide:methanol solution, dry 500 grams of formamide over 20 grams of anhydrous sodium sulfate for 24 hours);

(ii) Rinse the syringe, needle, and immediate container with two separate 5-milliliter portions of anhydrous methanol, in lieu of one 3-milliliter portion of anhydrous methanol; and

(iii) In paragraph (e)(3) of that section, add a sufficient volume of the formamide:methanol solution (2:1) to cover the electrodes in the dry titrating vessel, in lieu of 20 milliliters of solvent A before starting the titration.

(5) *Identity.* Using a solution containing 20 micrograms per milliliter of water and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the cefotiam working standard similarly tested.

(6) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[54 FR 20785, May 15, 1989]

§ 442.60 Cefpiramide.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefpiramide is (6*R*, 7*R*)-7-

[(*R*)-2-(4-hydroxy-6-methyl-nicotinamido)-2-(*p*-hydroxyphenyl)acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 974 micrograms of cefpiramide activity per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 9.0 percent.

(iii) Its pH in an aqueous suspension containing 5 milligrams per milliliter is not less than 3.0 and not more than 5.0.

(iv) Its total related substances content by high performance liquid chromatography is not more than 2.0 percent. No individual impurity is more than 0.7 percent.

(v) The specific rotation in dimethylformamide solution containing 10 milligrams of cefpiramide per milliliter is −106 ± 6 °C calculated on an anhydrous basis.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, total related substances, specific rotation, identity, and crystallinity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating to a wavelength of 254 nanometers, a 15- to 30-centimeter X 4-millimeter (inside diameter) column packed with microparticulate (5 to 10 micrometers in diameter) reversed phase packing material such as octylsilane bonded to silica, a flow rate not to exceed 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Reagents, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.01M phosphate buffer.* Dissolve 1.36 grams of monobasic potassium phosphate in 900 milliliters of water. Adjust the pH to 6.8 with 1*N* sodium hydroxide and dilute to 1,000 milliliters with water.

(B) *Mobile phase.* Mix 0.01*M* phosphate buffer: acetonitrile: tetrahydrofuran: methanol (880:40:40:40). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve and dilute an accurately weighed portion of the cefpiramide working standard in sufficient mobile phase to obtain a solution containing 0.25 milligram of cefpiramide activity per milliliter.

(B) *Sample solution.* Dissolve an accurately weighed portion of the sample in mobile phase and further dilute to 0.25 milligram of cefpiramide per milliliter (estimated).

(C) *Resolution test solution.* Dissolve an accurately weighed portion of cefpiramide working standard in 0.01*N* sodium hydroxide to obtain a solution containing approximately 1.0 milligram of cefpiramide activity per milliliter. Heat this solution at 95 °C for 10 minutes. This procedure allows cefpiramide lactone to be produced. Dilute 1.0 milliliter of this solution to 20 milliliters with mobile phase.

(iii) *System suitability requirements*)—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 0.95 and not more than 1.4.

(B) *Efficiency of the column.* From the number of theoretical plates (*n*) calculated as described in § 436.216(c)(2) of

this chapter calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:
L=Length of the column in centimeters;
n=Number of theoretical plates; and
d_p=Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 12.5 for the cefpiramide peak.

(C) *Resolution factor.* The resolution factor (R) between the peak for cefpiramide and the peak for cefpiramide lactone (generated in situ) is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_r in percent of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor (k′).* Calculate the capacity (k') for cefpiramide as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:
t_r=Retention time of cefpiramide in minutes; and
t_o=Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:
D=Column diameter in centimeters;
L=Column length in centimeters;
0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (k') for cefpiramide is satisfactory if it is not less than 2.0 and not more than 3.0. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of cefpiramide per milligram of sample as follows:

$$\text{Micrograms of cefpiramide per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:
A_u=Area of the cefpiramide peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the cefpiramide peak in the chromatogram of the cefpiramide working standard;
P_s=Cefpiramide activity in the cefpiramide working standard solution in micrograms per milliliter;
C_u=Milligrams of cefpiramide sample per milliliter of sample solution; and
m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 5 milligrams of cefpiramide per milliliter.

(4) *Total related substances.* Proceed as directed in paragraph (b)(1) of this section except use the following reagents, standard and sample solutions, and calculations:

(i) *Reagents*—(A) *0.03M phosphate buffer.* Dissolve 4.08 grams of monobasic potasssium phosphate in 800 milliliters of water. Adjust the pH to 7.5 with 1*N* sodium hydroxide and dilute to 1,000 milliliters with water.

(B) *Mobile phase.* Mix 0.03*M* phosphate buffer: methanol (750:250). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard and sample solutions.*

(A) *Working standard solution.* Transfer about 12.5 milligrams of 5-mercapto-1-methyl-1*H*-tetrazole (MMT) and an amount of cefpiramide working standard equivalent to about 25 milligrams of cefpiramide activity, both accurately weighed, to a 100-milliliter volumetric flask. Dissolve and dilute to volume with 0.03*M* phosphate buffer. Further dilute 2.0 milliliters of this solution to 100 milliliters with mobile phase.

(B) *Sample solution.* Transfer about 25 milligrams of the test material, accurately weighed, to a 50-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase.

(iii) *Calculations.* Calculate the percentages, individually, of MMT and any other compounds detected as follows:

$$T_1 = \text{Percent MMT (tetrazole)} = \frac{A_u \times C_s \times P_s \times 100}{A_s \times C_u \times 1{,}000}$$

$$T_2 = \text{Percent related compound} = \frac{R_u \times C_s \times P_s \times 100}{R_s \times C_u \times 1{,}000}$$

$$\text{L} = \text{Percent largest related compound} = \frac{L_u \times C_s \times P_s \times 100}{R_s \times C_u \times 1{,}000}$$

where:

A_u=Area of the tetrazole sample peak;

A_s=Area of the tetrazole working standard peak;

C_s=Concentration of the working standard in milligrams per milliliter;

P_s=Potency of the working standard in micograms per milligram;

C_u=Concentration of the sample solutions in milligrams per milliliter;

R_u=Sum of peak areas of other compounds, excepting MMT and cefpiramide, detected in the sample chromatogram.

R_s=Area of the cefpiramide working standard peak; and

L_u=Area of the largest related peak, except MMT.

T=Percent total related compounds=$T_1 + T_2$.

(5) *Specific rotation.* Dilute an accurately weighed sample with sufficient dimethylformamide to obtain a concentration of approximately 10 milligrams of cefpiramide per milliliter. Proceed as directed in §436.210 of this chapter, using a 1-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(6) *Identify.* Proceed as directed in §436.211 of this chapter using a 1-percent potassium bromide disc prepared as directed in §436.211(b)(1).

(7) *Chrystallinity.* Proceed as directed in §436.203(a) of this chapter.

[55 FR 14240, Apr. 17, 1990]

§442.69 Cefmetazole.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefmetazole is (6*R*,7*S*)-7-[2-[(cyanomethyl)thio]acetamido]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 970 micrograms of cefmetazole activity per milligram.

(ii) Its moisture content is not more than 0.5 percent.

(iii) It gives a positive identity test for cefmetazole.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §442.70a(b)(1).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Identity.* Proceed as directed in §436.211 of this chapter using a mineral oil mull prepared as described in paragraph (b)(2) of that section.

[59 FR 12546, Mar. 17, 1994]

§442.70a Sterile cefmetazole sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile cefmetazole sodium is the sodium salt of (6*R*-cis)-7-[[[cyanomethyl)thio]acetyl]amino]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is a lyophilized powder. It is so purified and dried that:

(i) If the cefmetazole sodium is not packaged for dispensing, its